

## Roundtable Discussion

# Research Implications of Pigment Biology in Zebrafish

Stephen C. Ekker,<sup>1</sup> David M. Parichy,<sup>2</sup> and Keith C. Cheng<sup>3</sup>

**Stephen C. Ekker:** We're here with Drs. Keith Cheng of the Pennsylvania State University Medical School at Hershey, Pennsylvania, and Dave Parichy of the University of Washington at Seattle, to have a roundtable discussion of pigment biology. They are the guest editors of this special issue of *Zebrafish*, which focuses on this topic. Dave and Keith, thanks for participating. To begin, can you each tell us how you got involved in the pigment biology of zebrafish?

**David M. Parichy:** For a long time I've been interested in pigment patterns, and I think one of the great things about pigment patterns in general is that you can approach them from so many different angles: whether you're interested in the genes and cell behaviors underlying pigment patterns or in the consequences of a particular pigment pattern in terms of the behavior of an organism or in the evolution of that pigment pattern. You can address pigment patterns at many different levels, constituting a sort of biological hierarchy. For a long time I had sought a system that would allow for the integration of such stratified information.

I began by studying amphibian pigmentation, but ended up switching to zebrafish primarily because of the opportunities for genetic studies, and especially forward genetics, with zebrafish, and the tremendous array of pigment patterns in species closely related to zebrafish. The extent of variation available for study is vast. Because of this, my laboratory has found pigmentation to be an abundant trait for study for the past several years.

**Stephen C. Ekker:** Keith, how did you get involved?

**Keith C. Cheng:** Serendipity, an interest in correlations between cellular structure and function, and most recently, an interest in the evolving role science has played and can play in the history of human societies. My research initially focused on the issue of somatic mutation as a critical cancer step.

In my search for a genetic screen in a vertebrate for somatic mutator, or genomic instability (*gin*) mutants, I used George Streisinger's simple assay for somatic mutation: the mosaic

eye assay. This simple assay uses the hypopigmented *golden* mutant, the very first mutant studied in zebrafish genetics. This simple assay uses *gol/+* heterozygotes to detect somatic mutations as pale retinal-pigmented epithelial cells (*gol/gol\**) in a background of black (*gol/+*) cells. Finding *gin* mutants and their cancer susceptibility left us with the need to identify the *golden* gene. That in turn led me to ask why the *golden* mutant is lighter in color than wild-type zebrafish. An ultrastructural comparison revealed something startling: lighter skin in both *golden* zebrafish and Europeans show a decrease in number, size, and pigment density of melanosomes. The *golden* gene turned out to be a new pigmentation gene, *slc24a5*, or *nckx5*, governing sodium–calcium exchange. Analysis of the online database of human genetic variation, the HapMap, together with studies of human populations of mixed ancestry, allowed us to conclude that the human ortholog had undergone an adaptive mutation during the evolution of the light skin color in Europeans. But that's just the first half of the human pigmentation story—we still need to know what is responsible for the light skin of East Asians/Amerindians! The huge social consequences of the "scientific" connections drawn between skin color and "race," together with the potential to contribute to society's understanding of skin color and our common ancestry, have now drawn me professionally into an entirely new field.

**Stephen C. Ekker:** Between the two of you, then, we have what might be called the two ends of the spectrum of zebrafish pigment biology. And that realm of zebrafish study, numbering many investigations, dates far back into the history of the field.

Can you each name some of the papers that have been the most influential in affecting the way you look at zebrafish pigment biology?

**Keith C. Cheng:** I look at fish pigmentation biology largely through my new interest in mechanisms of normal human pigmentation. One of the most striking papers<sup>1</sup> was actually out of the medaka genetics literature. This paper describes the

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identification of a membrane-associated transporter protein gene, *MATP* (also known as *AIM1*). Concurrent work from the Brilliant Lab<sup>2</sup> drew a link between the same gene in mouse, called *underwhite*, and human oculocutaneous albinism type 4. Even later work led to the even more exciting finding that a coding SNP in this gene contributed to the pigmentary difference between West Africans and Europeans. How cool that the cloning of the gene in medaka fish used variants collected over several hundred years in Japan.<sup>3,4</sup> I surmise that further significant contributions to understanding of pigmentation mechanisms will come from other medaka and zebrafish pigmentation mutants, particularly those that affect the degree, rather than pattern of pigmentation. I think it's great that serendipitous links between the medaka B mutants, the zebrafish *golden* mutation, and evolutionary origins of human skin color variation together cement the validation of fish as models for studying processes related to humans.

**David M. Parichy:** Some of the papers that I found most influential were the early papers of Victor Twitty at Yale, published in the 1930s, which argued for the potentially great utility of pigment patterning as a model system. I came across these papers while a graduate student and originally looking at the pigmentation system. They were reports of studies of salamander pigmentation, and through these papers Twitty presented a superb early embryological dissection of how pigment patterns form, and made an early effort to understand how pattern differences arose between species.

For the most part, this was really cut-and-paste embryology, and some of it turned out not to be quite right. But I think that most of what Twitty found was right.

But the question was how to take this system into the modern molecular era. For that you really want to be able to do genetic studies. In this latter regard, one of the more recent papers that was very influential in my decision to work on the pigmentation system in the first place was written a number of years ago by Steve Johnson of Washington University in St. Louis, and was published in *Developmental Biology*.<sup>5</sup> It showed how one could use a genetic approach to dissect not only genetic pathways, but also various cell populations that participate in generating an adult pigment pattern. That was one of the major factors in convincing me that where the genetic resources are available, using zebrafish would provide a highly tractable system for the study of pigment biology, with a lot of potential for studies in many different directions.

I would therefore have to say that those two bodies of work, separated by almost 70 years, were probably the most influential in terms of getting me into study of the pigmentation system with zebrafish.

**Stephen C. Ekker:** I also love those old papers, especially the ones published in the 1920s and '30s and some in the 1940s. They really present beautiful science and a rich history for our work. But what sort of shape will the future have? What do you see as the big challenges and opportunities in the field of pigment biology, and where can we go now with the beautiful set of tools and rich scientific body of knowledge that we now have in this field?

**Keith C. Cheng:** One of the most exciting big new challenges and opportunities is to use pigmentation biology as a

model for the study of gene function. It has a richness that involves chemistry, biochemistry, genetics, reverse genetics, genomics, and quantitative measurements of pigmentation. In the field of systems genetics, the zebrafish is one of a number of organisms that can be used to address problems related to human health. But one of the most striking features about the use of zebrafish, which was apparent from the zebrafish Atlas Project, is the potential for taking advantage of their small size as whole vertebrate organisms while being able to obtain the highest possible resolution, even at the subcellular level, in studying processes such as melanosome morphogenesis in pigmentation. Studies of this kind can certainly be done in cell culture, but if you want to look at some of these events *in situ* in the context of a real organism, zebrafish and medaka present real opportunities for advancement in our knowledge of pigment biology. We will, with any luck, see the day when zinc-finger nucleases allow us to insert any change virtually anywhere in the genome. New imaging technologies such as nano-computed tomography and laser sheet confocal technology will allow us to study morphological changes at unprecedented resolutions. The ability to connect three-dimensional imaging techniques with the power of forward and reverse genetics will allow us to answer questions of biological function and mechanism in exciting new ways. I leave Dave to comment about zebrafish and the study of melanoma.

**Stephen C. Ekker:** Dave, where do you see us going?

**David M. Parichy:** If you look at the articles already published in zebrafish pigment biology, it's clear that individual laboratories can pursue studies in a wide array of directions. A case in point would be the review by Craig Ceol and other researchers in Leonard Zon's laboratory at the Howard Hughes Medical Institute. It's very clear that this is an incredibly powerful system for looking at the genomic alterations that underlie tumor formation and metastasis, for other pathophysiologic processes, and for drug discovery. And you can go all the way from the sort of basic biology that my laboratory does in the study of melanoma and bring that sort of paradigm all the way to translational research and to the point where in 5 or 10 years, research on zebrafish pigment biology can lead to clinical trials.

That indicates a very clear trajectory from zebrafish biology to clinical medicine. If you look at some of the other reported work in pigment biology, it is also clear that the zebrafish provides a powerful system for understanding stem cell biology and regeneration. And I think there will also be major advances along those lines.

I think the big challenge, however, will be to integrate methods across different levels of the biological hierarchy, such as taking what we know about how variant forms arise in zebrafish as a result of single- or double-locus mutation and extending that to the manner in which variation arises in natural populations. And by extension, how that variation in turn affects behavior, ecology, and species interactions.

And that leads back to zebrafish pigment biology because it provides outstanding potential for integrative studies, and some of the greatest promise lies in translational biology.

With regard to current issues, I think we need a better understanding of how pigment pattern affects zebrafish fitness in the field, to take but one example, and also of the way

in which different populations of zebrafish or their relatives have undergone changes over time at the genomic level as a result of selection or drift, and the phenotypic consequences of those changes. Clearly, those are things that will have to involve an integration of field biology, in terms of the natural history of the organism, with both large-scale genomic biology and novel approaches to morphogenesis, and with the ways in which genomic alterations are translated into specific cellular and morphological outcomes.

So I think there is great potential in zebrafish pigment biology not only for individual research programs that are just getting started, but also for bringing together what are currently very disparate research programs. Hopefully, this issue of *Zebrafish* will facilitate communication between laboratories that may have very different research specializations but would be interested in working together toward this kind of goal.

**Stephen C. Ekker:** Keith, did you have anything that you wanted to add?

**Keith C. Cheng:** I'd like to emphasize that the zebrafish pigment cell, in being visible, allows one to study biological problems as disparate as pattern formation and tumor development. That visibility allows us to directly visualize cancers of pigmented cells like melanoma, and to detect, indirectly, events in the genome that result in changes in the expression of genes that affect color. Pigmented cells in fish allow us to study fundamental issues of cell differentiation, including epigenetic control of gene expression. In short, the pigmented cell is a great biological "light bulb" that can illuminate a host of genetic and cellular mechanisms.

Finally, history obligates us to note that work on skin pigmentation relates to the most notable phenotype associated with race—skin color. This presents us with a responsibility to take special care to ensure that this area of research is represented accurately and that we do not repeat the historical mistake of some of our scientific predecessors of allowing tribal instincts to cloud our thinking about biology. It is tre-

mendously exciting that a properly planned dissemination of scientific understanding of how we differ in skin color may be used to put to rest hundreds of years of myth that has associated skin color directly with human worth.

**Stephen C. Ekker:** Thank you, gentlemen. I think this issue of *Zebrafish* will represent a valuable package for the research community, and we're hoping that it will be read not only by pigment biologists and other zebrafish investigators, but also by everyone from oncologists to ecologists.

## References

1. Fukamachi S, Shimada A, Shima A. Mutations in the gene encoding B, a novel transporter protein, reduce melanin content in medaka. *Nat Genet* 2001;28:381–385.
2. Newton JM, Cohen-Barak O, Hagiwara N, Gardner JM, Davisson MT, King RA, *et al.* Mutations in the human orthologue of the mouse underwhite gene (*uw*) underlie a new form of oculocutaneous albinism, OCA4. *Am J Hum Genet* 2001;69:981–988.
3. Aida T. On the inheritance of color in a fresh-water fish, *Aplocheilichthys latipes* Temmick and Schlegel, with special reference to sex-linked inheritance. *Genetics* 1921;6:554–573.
4. Kelsh RN, Inoue C, Momoi A, Kondoh H, Furutani-Seiki M, Ozato K, *et al.* The Tomita collection of medaka pigmentation mutants as a resource for understanding neural crest cell development. *Mech Dev* 2004;127:841–859.
5. Johnson SL, Africa D, Walker C, Weston JA. Genetic control of adult pigment stripe development in zebrafish. *Dev Biol* 1995;167:27–33.

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